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Erysiphe magnoliicola, a new powdery mildew on *Magnolia*

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Abstract — A new powdery mildew species of *Erysiphe* sect. *Microsphaera*, collected on three species of *Magnolia*, is here described, illustrated, and named as *E. magnoliicola* sp. nov. Introduction of a new species is supported by phylogenetic analyses of ITS and 28S rDNA of Korean and Japanese collections of this fungus. A comparison of and a synoptic key to the four species of *Erysiphe* sect. *Microsphaera* known on *Magnolia* spp. are provided.

Key words — *Erysiphaceae, Erysiphe magnifica, Magnoliaceae*, molecular phylogeny

Introduction

Powdery mildews (*Erysiphaceae*, *Ascomycota*) are plant pathogenic fungi that are holobiotrophic parasites on leaves, stems, flowers, and fruits. These pathogens cause moderate to serious damage on nearly 10,000 species of angiosperms (Braun et al. 2002, Glawe 2008). Braun (1987) introduced a classification of the *Erysiphaceae* that split the family into 18 genera, including *Erysiphe*, *Microsphaera*, and *Uncinula*. Later Braun & Takamatsu (2000) reduced the genera *Microsphaera* and *Uncinula* to synonymy with *Erysiphe* s. lat. based on phylogenetic analyses and proposed two new non-phylogenetic sections: *E.* sect. *Uncinula* (Lév.) U. Braun & Shishkoff and *E.* sect. *Microsphaera* are characterized by having chasmothecial appendages with dichotomously branched tips.

Three species of *Erysiphe* sect. *Microsphaera* — *E. magnifica* (U. Braun) U. Braun & S. Takam., *E. magnoliae* (Sawada) U. Braun & S. Takam., and *E. bulbosa* (U. Braun) U. Braun & S. Takam. — have been recorded on *Magnolia* spp. *Erysiphe magnifica* is a well-known species infecting *Magnolia* species in North and South America, eastern Asia, and Europe (Braun 1987 & 1995,

Glawe 2003, Braun & Cook 2012, Farr & Rossman 2014). *Erysiphe magnoliae* is known from Japan and Korea as the causal agent of powdery mildew on *M. obovata* (Nomura 1997, Braun & Cook 2012, Takamatsu et al. 2013). This species is clearly distinguished from *E. magnifica* by having conidiophore foot-cells with inflated bases, and the status of *E. magnoliae* as a separate species has recently been confirmed by molecular data (Takamatsu et al. 2013). *Erysiphe bulbosa*, endemic to China, was recorded on *M. liliiflora* (Braun 1988; Wang 1987). This fungus can be differentiated from *E. magnifica* and *E. magnoliae* by having chasmothecial appendages with a distinct swelling at the base.

During a field survey of phytopathogenic fungi carried out after the publication of comprehensive descriptions of powdery mildews in Korea (Shin 1988, 2000), a new powdery mildew species was found on *Magnolia* species. In a previous study, it was suggested that *E. magnoliae* has unique characteristics compared to other powdery mildew species described on *Magnolia* (Takamatsu et al. 2013). However, this study, which focused on *E. magnoliae,* was not a comprehensive study of *Magnolia* powdery mildews.

We describe our new species found in Korean and Japanese specimens based on morphological characters and molecular analyses and discuss relationships with closely related powdery mildew fungi infecting *Magnolia* species.

Materials & methods

The fungus was detached from the infected leaves and mounted in a few drops of distilled water on a glass slide for light microscopy. The morphology of the fungal structures was examined in bright field and differential interference contrast (DIC) light microscopy, using an Olympus BX51 microscope (Olympus, Tokyo, Japan) for measurements and a Zeiss AX10 microscope equipped with AxioCam MRc5 (Carl Zeiss, Göttingen, Germany) for photographs. The measurements were performed at 100×, 200×, 400×, and 1000× magnifications. Voucher specimens were deposited in the Korea University Herbarium (KUS), Korea; and the Mie University Mycological Herbarium (MUMH), Japan.

Genomic DNA was extracted from chasmothecia using the Chelex 100 as previously described (Walsh et al. 1991, Hirata & Takamatsu 1996). The 28S rDNA (including domains D1 and D2) and internal transcribed spacer (ITS) rDNA (ITS1, 5.8S, ITS2) regions were amplified by the polymerase chain reaction (PCR), and then sequenced directly (Takamatsu et al. 2009). The sequences were edited using the DNASTAR computer package version 5.05 (Lasergene, Madison, WI, USA) and resulting sequences were deposited in GenBank under the accession numbers of KJ567065–KJ567075. Additional sequences of *Erysiphe* spp. were retrieved from GenBank. Alignments were done with an online version of MAFFT 7 (Katoh & Standley 2013). Maximum likelihood analyses were performed using PhyML 3.0 (Guindon & Gascuel 2003). The best fitting substitution models were determined with jModelTest 2.1.4 (Darriba et al. 2012). The branches of the inferred tree were tested by the bootstrap analysis of 1000 replicates. The phylogenetic tree generated from the ITS analysis was deposited in TreeBASE (http://www.treebase.org/) under the accession number of S15516.

Taxonomy

Erysiphe magnoliicola S.E. Cho, S. Takam. & H.D. Shin, **sp. nov.** Fig. 1

MycoBank MB 808247

Differs from *Erysiphe magnifica* by its longer conidiophore foot-cells and its smaller number of chasmothecial appendages and from *E. magnoliae* by its conidiophore foot-cells with a cylindrical base.

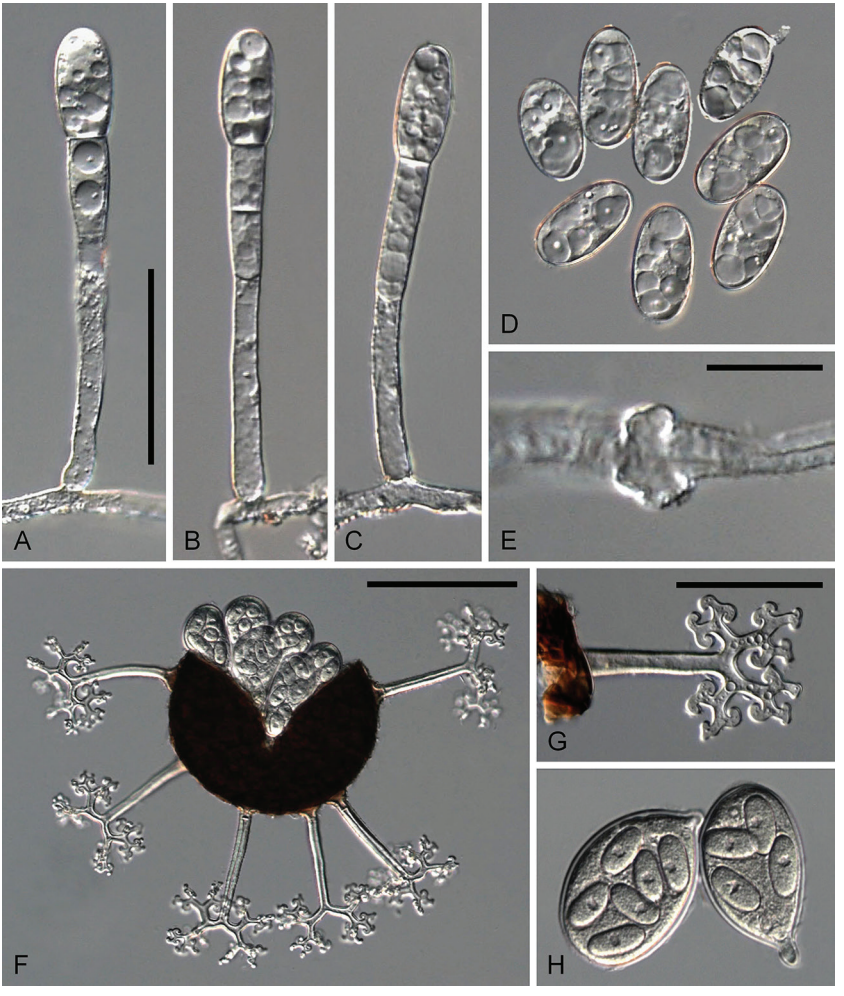

Fig. 1. *Erysiphe magnoliicola*. A–C: Conidiophores. D: Conidia. E: Appressoria. F: Surface view of a conidium showing angular/rectangular wrinkling pattern. G: Chasmothecium containing several asci. H: Appendage. I: Asci containing 4–6 ascospores. Scale bars: A–D, H–I = 50 µm; E–F = 10 µm; G = 100 µm.

Type: 38°11′31″N 128°21′44″E, on *Magnolia sieboldii* K.Koch (*Magnoliaceae*), 24 September 2012, H.D. Shin & S.E. Cho (**Holotype**, KUS-F26977; GenBank KJ567072, KJ567065).

Etymology: The epithet is derived from the host plant genus.

Colonies amphigenous, thinly effused or conspicuous, forming circular to irregular white patches. Hyphae straight or almost so, hyaline, septate, branched, 4–8 µm wide. Hyphal appressoria well developed, moderately lobed to multi-lobed, solitary or in opposite pairs. Conidiophores arising from the upper part of mother cells, 85–137 × 8.5–10 µm, producing conidia singly, followed by 2 to 3 cells. Foot-cells mostly straight or almost so, slightly sinuous at the base, relatively long, 42.5–70 µm. Conidia formed singly, hyaline, ellipsoidal to oval, 32.5–42.5 × 17.5–21.3 µm with a length/width ratio of 1.6–2.5, lacking fibrosin bodies, producing germ tubes on perihilar position, with an angular/rectangular wrinkling pattern on the surface. Chasmothecia amphigenous, mostly hypophyllous, cauligenous, gregarious, dark brown, spherical, 100–140 µm diam., containing 4–7 asci. Peridium cells irregularly polygonal or rounded, 10–25 µm wide. Appendages equatorial, 6–10, mostly aseptate, rarely 1-septate, 4–5 times regularly dichotomously branched, 0.5–1 times as long as the chasmothecial diam., 70–100 × 7.5–9 µm, tips distinctly recurved, brown at the base, becoming paler toward the tip. Asci obovoid to ellipsoid or saccate, mostly short-stalked, 57.5–70 × 40–45 µm, containing 3–6 spores per ascus. Ascospores hyaline, oblong-ovoid, 20–27.5 × 10–12.5 µm.

Additional specimens examined –

On *Magnolia sieboldii*: **KOREA**, **Pyeongchang**, Byeongnae-ri, 13 Sep. 1999, H.D. Shin (KUS-F16655); **Hongcheon**, Bukbang-myeon, 29 Sep. 2000, H.D. Shin (KUS-F17710); 17 Sep. 2001, H.D. Shin (KUS-F18474); 11 Oct. 2002, H.D. Shin (KUS-F19252); 9 Sep. 2007, H.D. Shin (KUS-F22848); Nae-myeon, 20 Aug. 2012, H.D Shin & S.E. Cho (KUS-F26833); **Suwon**, Seodun-dong, 5 Nov. 2004, H.D. Shin (KUS-F20960); 20 Sep. 2005, H.D. Shin (KUS-F21395); **Namyangju**, Experimental Farm of Korea University, 19 Oct. 2005, H.D. Shin (KUS-F21546; GenBank KJ567072, KJ567065); Sudong-myeon, 13 Aug. 2012, H.D. Shin & S.E. Cho (KUS-F26807); **Gangneung**, Seongsan-myeon, 29 Aug. 2011, H.D. Shin & S.E. Cho (KUS-F26035); 3 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27041); **Gapyeong**, Seolak-myeon, 23 Aug. 2012, H.D. Shin & S.E. Cho (KUS-F26843); **Inje**, Yongdae-ri, 24 Sep. 2012, H.D. Shin & S.E. Cho (KUS-F26977); **Gyeongju**, Forest Environment Research Institute, 8 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27077).

On *Magnolia liliiflora* Desr.: **KOREA**, **Suwon**, Seodun-dong, 17 Oct. 1997, H.D. Shin (KUS-F14447); 4 Oct. 1999, H.D. Shin (KUS-F16918); 16 Oct. 2008, H.D. Shin (KUS-F23831); 19 Oct. 2011, H.D. Shin & S.E. Cho (KUS-F26353); 14 Aug. 2012, H.D. Shin & S.E. Cho (KUS-F26821); 11 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27100); **Jinju**, Gajwa-dong, 15 Oct. 2003, H.D. Shin (KUS-F19903; GenBank KJ567074, KJ567069); 1 July 2004, H.D. Shin (KUS-F20383); **Jeju**, Jeju-si, 13 Nov. 2003, H.D. Shin

(KUS-F20074); 23 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27154); **Gyeongju**, Forest Environment Research Institute, 17 Jun. 2011, H.D. Shin (KUS-F25786); **Seoul**, Korea Forest Research Institute, 13 Oct. 2012, H.D. Shin & S.E. Cho (KUS-F27111; GenBank KJ567073, KJ567067).

On *Magnolia denudata* Desr.: **JAPAN, Gifu Prefecture**, Sekigahara-cho, 8 Nov. 2011, S. Takamatsu & J. Meeboon (MUMH5501; GenBank KJ567075, KJ567068).

Phylogenetic analyses

Additional specimens sequenced – ***Erysiphe magnifica***: **JAPAN**, **Aichi Prefecture**, Nagoya-shi, Higashiyama Botanical Garden, on *Magnolia liliiflora*, 13 Oct. 2011, S. Takamatsu & J. Meeboon (MUMH5353; GenBank KJ567070, KJ567066). On *Magnolia* sp.: **ARGENTINA**, **Buenos Aires**, Japanese Garden, on *Magnolia* sp., 12 Mar. 2004, S. Takamatsu (MUMH2539; GenBank KJ567071).

Based on the molecular phylogenetic trees generated from the PhyML analyses, sequences generated from four isolates of *E. magnoliicola* (the Korean holotype, two other Korean isolates, and one Japanese isolate) formed an independent ITS clade with 99% bootstrap support, and an independent 28S clade with 87% bootstrap support (Figs 2–3). This lineage was separated from *E. magnoliae* and *E. magnifica* in ITS and 28S trees. Both phylogenetic analyses clearly support *E. magnoliicola* as an independent species.

Discussion

Erysiphe magnifica, described from the United States, has been recorded on *Magnolia* spp. throughout the world, including Korea and Japan (Braun & Cook 2012, Farr & Rossman 2014). It has also been found on lotus, *Nelumbo nucifera* Gaertn. (*Nelumbonaceae*), in a glasshouse in Germany and the identity of this unusual collection has been confirmed based on morphological characteristics and molecular sequence analysis (Kirschner 2010). *Erysiphe magnifica* differs from *E. magnoliicola* by its shorter conidiophore foot-cells (15–45 µm) and its more numerous chasmothecial appendages (5–23; Braun & Cook 2012). *Erysiphe magnoliae,* which has been reported on *M. obovata* in Korea and Japan, is clearly distinguished from *E. magnoliicola* by having conidiophores with shorter (15–25 µm) basally inflated foot-cells (Takamatsu et al. 2013). *Erysiphe bulbosa* is quite distinct from other species on *Magnolia* spp., and unique in having chasmothecial appendages with swollen bases (Braun 1988); the anamorph of *E. bulbosa* is still unknown.

Although *E. magnoliicola* is phylogenetically close to *E. magnoliae,* the sequence analyses (Figs 2–3) clearly distinguish two species and support the hypothesis that they have diverged from a common ancestor; *E. magnoliae* is sister to the *E. magnoliicola* clade with BS values of 89% (ITS) and 82% (28S). *Erysiphe magnifica,* which is not closely related to *E. magnoliae* and *E. magnoliicola*, may have developed independently from a separate lineage.

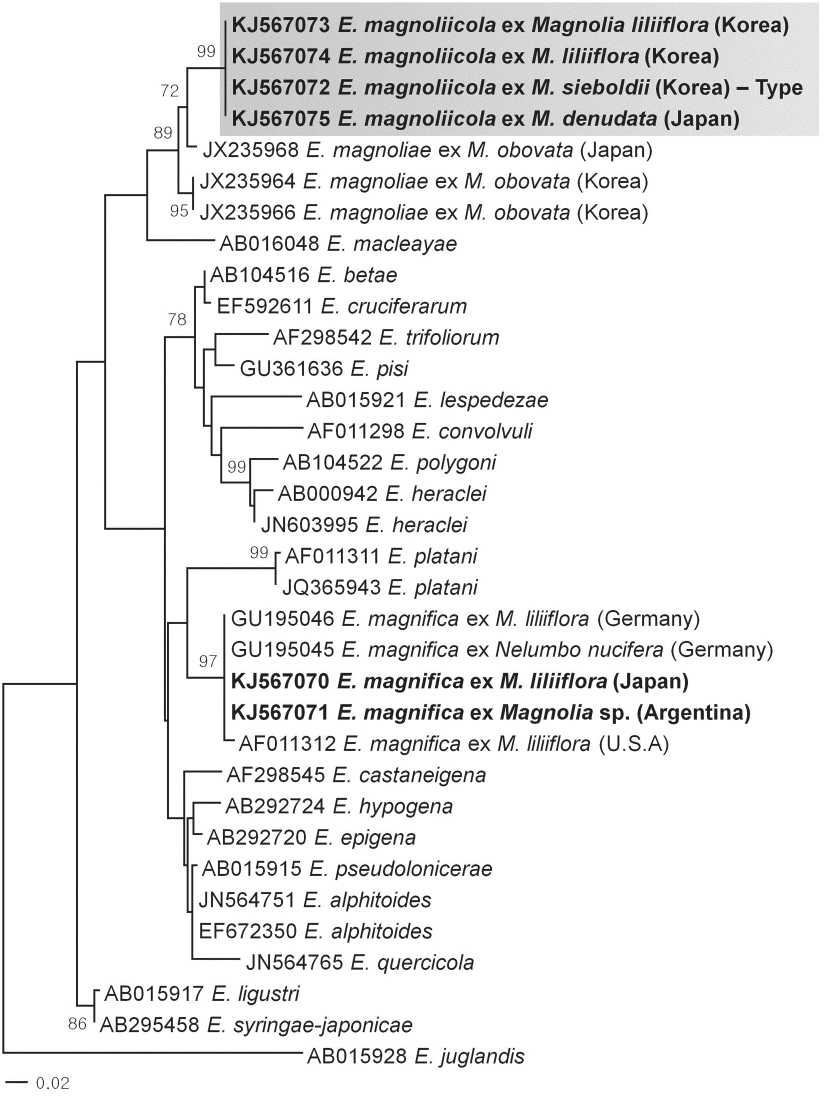

Fig. 2. Phylogenetic relationship between *Erysiphe* sequences and some reference isolates retrieved from GenBank, inferred by PhyML using the ITS rDNA region. Numbers above the branches represent the bootstrap values of over 70% obtained from 1000 bootstrap replicates. New sequences are indicated with bold font. Bar = number of nucleotide substitutions per site.

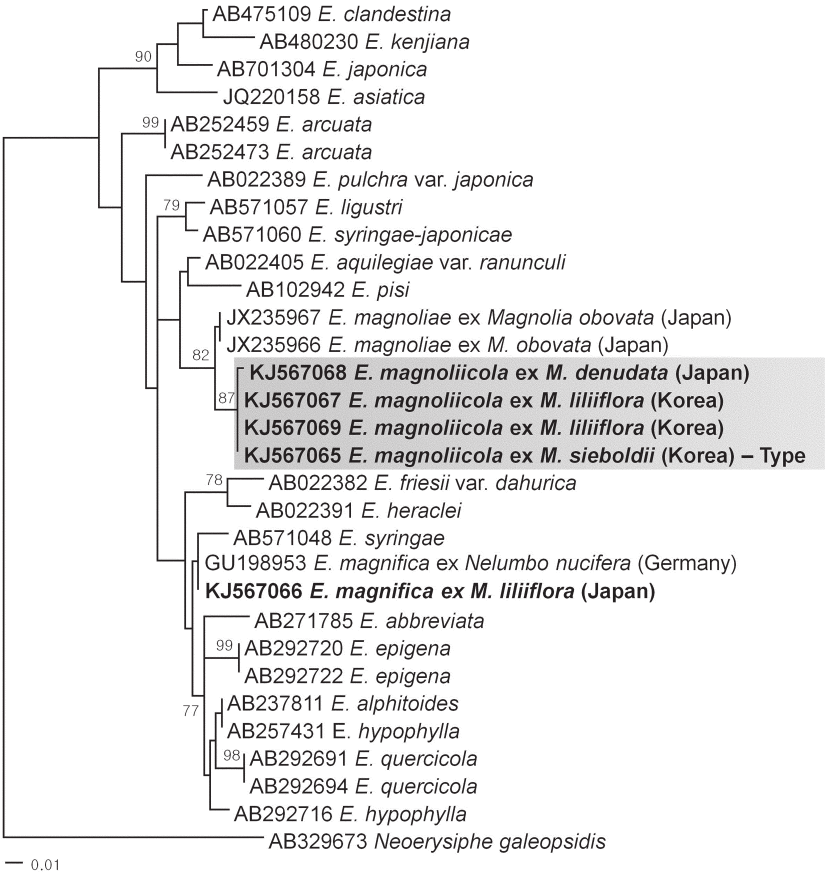

Fig. 3. Phylogenetic relationship between *Erysiphe* sequences and some reference isolates retrieved from GenBank, inferred by PhyML using the 28S rDNA region. Numbers above the branches represent the bootstrap values of over 70% obtained from 1000 bootstrap replicates. New sequences are indicated with bold font. Bar = number of nucleotide substitutions per site.

Molecular analyses of *E. bulbosa* have not yet been performed and are urgently required to address the phylogenetic relationships of powdery mildews on *Magnolia*.

Magnolia is a large genus, with about 210 species in *Magnoliaceae* subfam. *Magnolioideae,* including *M. liliiflora* and *M. denudata* native to China and *M. sieboldii* native to eastern Asia (China, Japan, and Korea; Azuma et al. 1999). These species are planted as ornamental trees throughout the world. Sequences

KJ567070 (Fig. 2) and KJ567066 (Fig. 3) came from a specimen of *E. magnifica* on *M. liliiflora* collected in the American Magnolia Garden in Higashiyama Botanical Garden, Nagoya, Japan. It has been suggested that the tree might have been imported from the United States and planted in the Japanese garden for ornamental purpose. ITS sequences retrieved from *E. magnifica* collections on *M. liliiflora* in North America, Argentina, and Germany and on *N. nucifera* in Germany have already been reported (Fig. 2). *Erysiphe magnifica* has been recorded on four *Magnolia* species (*M. kobus*, *M. obovata*, *M. liliiflora*, and *M. sieboldii)* in Korea (Shin 2000, Korean Society of Plant Pathology 2009). Although Lee (1975) recorded *E. magnifica* on *M. kobus,* no herbarium material has been preserved, and one of us (HDS) has not found any powdery mildews on *M. kobus* during his over 30 years' of field-work in Korea. Therefore, presence or identity of *E. magnifica* on *M. kobus* in Korea is obscure. Recently, powdery mildew caused by *E. magnoliae* (previously known as *M. magnifica* in Korea) on *M. obovata* in Korea and Japan was re-examined morphologically and phylogenetically (Takamatsu et al. 2013). We introduce *Erysiphe magnoliicola* as a new species on *M. liliiflora* and *M. sieboldii* in this study and confirm *E. magnoliae* and *E. magnoliicola* as causative agents of powdery mildews on *Magnolia* spp. in East Asia.

Key to species of *Erysiphe* sect. *Microsphaera* on *Magnolia* spp.

1. Chasmothecial appendages distinctly swollen at the base ..... ***E. bulbosa***
1. Chasmothecial appendages not swollen at the base **2**
2. Foot-cells of conidiophores inflated at the base ..... ***E. magnoliae***
2. Foot-cells of conidiophores not inflated at the base **3**
3. Foot-cells of conidiophores relatively short (15–40 µm); chasmothecia with 5–23 chasmothecial appendages ..... ***E. magnifica***
3. Foot-cells of conidiophores relatively long (42.5–70 µm); chasmothecia with 6–10 chasmothecial appendages ..... ***E. magnoliicola***

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